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PRINCIPAL INVESTIGATOR: Hsieh, Jer-Tsong

CONTRACTING ORGANIZATION: UT Southwestern Medical Center
5323 harry Hines Blvd., Dallas, TX 75390

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Jer-Tsong Hsieh

E-Mail: jt.hsieh@utsouthwestern.edu

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14. ABSTRACT

Conventional chemotherapy with cell killing en mass often targets mitotic cells with less specificity, which likely leads to undesirable side effect. Knowing specific molecular defects in cancer cells has led to discover new chemotherapeutic agents. Thus, combined agents targeting different defected pathways in cancer cells have a better chance to eradicate tumor completely. Thus, to achieve a cure, a comprehensive targeting strategy needs to be implemented. In addition, improved methods for monitoring drug delivery and tumor response in a nearly real-time manner should offer a safe and effective treatment. This project carried out by a team of chemist, radiologist, and molecular tumor biologist is to develop a novel drug delivery system with new small molecular therapeutic agents assisted with new imaging probe is expect to bring a new frontier for prostate cancer management. Our objective is to develop dendrimer-based theranostic agent with prostate cancer specificity and positron emission tomography imaging capability that can prevent the early onset of PCa metastasis or delay the progression of metastasis. The mission of my project is to design small peptide derived from tumor suppressor DAB2 family as therapeutic agent and examine its biology activities.

15. SUBJECT TERMS

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INTRODUCTION

Increasing evidence has indicated the role of cancer stem cell (CSC) in cancer progression towards more aggressive cancer. CSCs share many characteristics with somatic stem cells, such as immortal and self-renewal. In addition to normal stem cell properties, CSCs appear to be tumor–initiating and resistant to therapies because of their quiescence. Increasing evidence indicates that the presence of CSC in the end stage of disease (1). Although the cell origin of CRPC remains controversial, several studies clearly indicate the presence of CSC in CRPC (2, 3). Clinical evidence suggests that an increased CSC in tumor mass may contribute to the failure of conventional therapies. Castration resistant prostate cancer (CRPC) recognized as a lethal disease has been implied to derive from stem cell population associated with its resistance to anti-androgen therapy and chemotherapy.

Targeted therapy now becoming an active research area of cancer therapy is expected to achieve better efficacy for individual patient. In order to target prostate cancer (PCa) specifically, we are developing a novel cancer cell-selective dendrimer nanoparticle platform with both targeted imaging and drug delivery capabilities to target metastatic PCa. Using this unique delivery system, we expect be able to monitor the drug delivery and/or response of cancer cells in a real-time manner. Our major activities are constructing dendrimer conjugated with therapeutic peptide, determining the mechanism of action and preparing chelator for conjugating PET tracer and dendrimer.

BODY

From our previous data, we were able to identify the most potent small peptide conjugated with dendrimer unit. In order to ensure the activity resulted from small peptide in this conjugate, Dr. Simanek has constructed a variety of control peptides for further testing this year. In addition, using this most potent conjugate, we also perform the *in vivo* biochemical activities by implanting minipump containing this conjugate into tumor site.

Aim 4: To evaluate the therapeutic efficacy using various pre-clinical models

Task 8 (Months 25– 36) Effect of dendrimer-conjugates.

Although we obtained some promising results from the P10 therapeutic peptide conjugated with dendrimer unit (CSIV-81) from last year. Before studying pharmacokinetics, bio-distribution and therapeutic efficacy in xenograft model, we decided to compare the control therapeutic peptide (i.e., CSVI-24, -29, and CVS-88) and another therapeutic peptide (i.e., CSIV-78) in the unit by adding targeting peptide R11. We tested a variety of conjugates for their cytotoxicity and effect on inhibiting AKT and Src activation (i.e., phosphorylation) (Fig. 1).

From the time course study (Fig. 1A), CSIV-81 remained very active throughout 72 hrs after treatment. However, overall results appeared not very consistent. For example, CSIV-78 showed no cytotoxicity (Fig. 1B) as well as no inhibition on AKT and Src

activation (Fig. 1C). Also, CSVI-24 and still showed significant toxicity in cells (Fig. 1B). We therefore are working on change chemical design to generate better compound.

Nevertheless, we went ahead to deliver CSIV-81 into animal using minipump to examine its potential efficacy. Initially, we had some problem for animal model because source of animal was infected. So, we have to delay for sometime until the colony reestablished. In the first experiment, we delivered 10 mg/kg of CSIV-81, due to the limitation from the synthesis, into animal bearing PCa cells and examined its effect on AKT inactivation based on phosphorylation pattern. However, the negative result was obtained (Fig. 2). Now, we are doing dose escalation in hope to reach effective dosage.

KEY RESEARCH ACCOMPLISHMENTS

- Validate the activities of dendrimer unit conjugates.
- One joint publication describing Click-chemistry for labeling antibodies with Copper-64 as a PET imaging agent.

REPORTABLE OUTCOMES

- Amit, K., Hao, G., Li, L., Ramezani, S., Hsieh, J.T., Oz, O., Sun, X. (2015) Click-chemistry Strategy for labeling antibodies with Copper-64 via a cross-bridged tetraazamacrocyclic chelator scaffold. Bioconjugate Chem. (In press).

CONCLUSION

In the third year, we have tested more dendrimer conjugates including control peptides for their activities; the activities of these conjugates were not consistent. Also, we did not observe any enhancement of activity after dendrimer conjugation and the activity in animal is not observed. Further synthesis is under way in hope to solve these problems.

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3. Vander Griend DJ, Karthaus WL, Dalrymple S, Meeker A, DeMarzo AM, Isaacs JT. The role of CD133 in normal human prostate stem cells and malignant cancer-initiating cells. Cancer Res. 2008;68:9703-11.

Appendices

Table 1 The structure and composition of dendrimer unit conjugates.

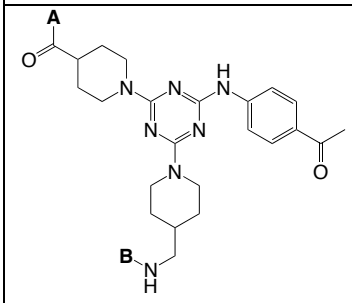
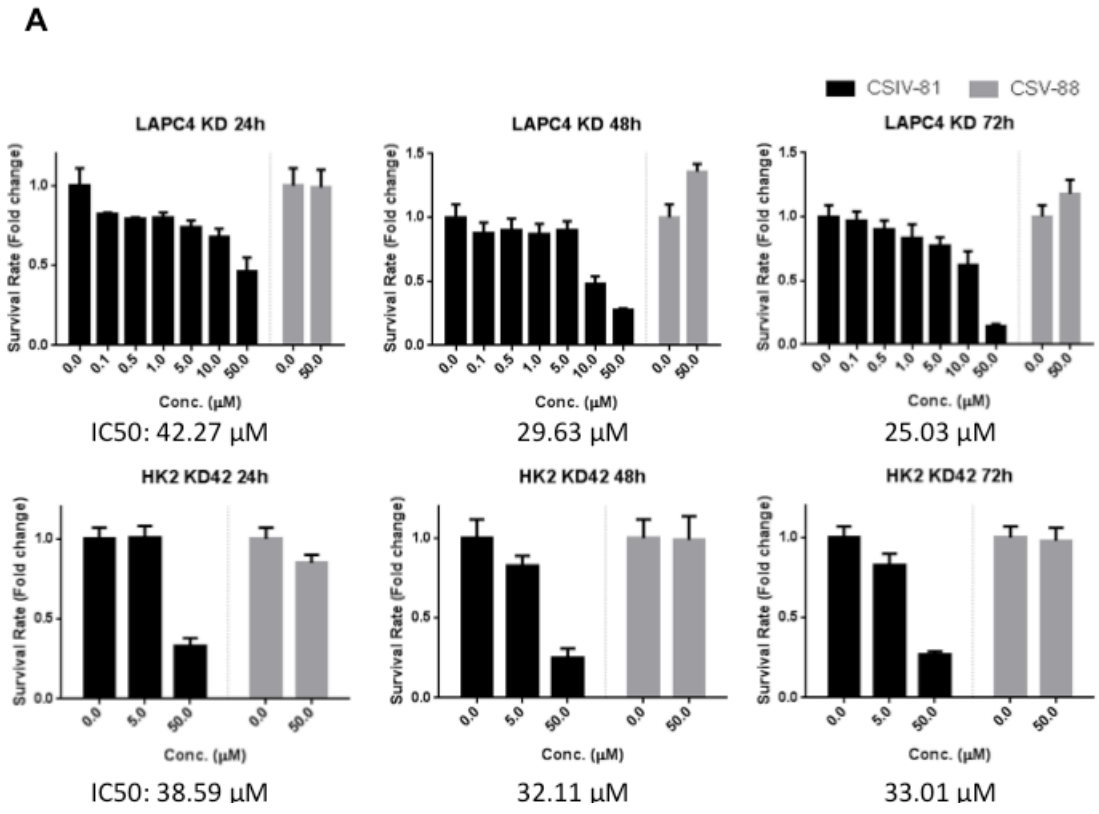
Skeleton	Name	A	B
	CSV-88	OH	H
	CSVI-29	FQLRQPPLVPSRKGEG(OH)	H
	CSVI-24	G(OH)	$(H_2N)R_{11}$
	CSVI-27	FQLRQPPLVPSRKGEG(OH)	$(H_2N)R_{11}$
	CSVI-30	FQLRQAALVASRKGEG(OH)	$(H_2N)R_{11}$
	CSIV-81 (original)	FQLRQAALVASRKGEG(OH)	$(H_2N)R_{11}$
	CSIV-78	$P_{10}G(OH)$	$(H_2N)R_{11}$

Figure 1 The activities of dendrimer-PR peptide in PCa cells. (A) Either LAPC4 KD or HK2 KD (3000 cells/well) was treated with different concentrations of conjugates (CSIV-81, CSV-88) for different time then relative cell number was determined by MTT assay. (B) Either LAPC4 KD or HK2 KD42 no (3000 cells/well) was treated with different concentrations of conjugates (CSIV-81, CSVI-27, CSVI-24, CSVI-30, CSIV-78, CSVI-29) for 24 hrs then relative cell number was determined by MTT assay. (C) Cells were treated with 50 mM of peptide 30 min and cell lysates were harvested and subjected to western blot analyses.



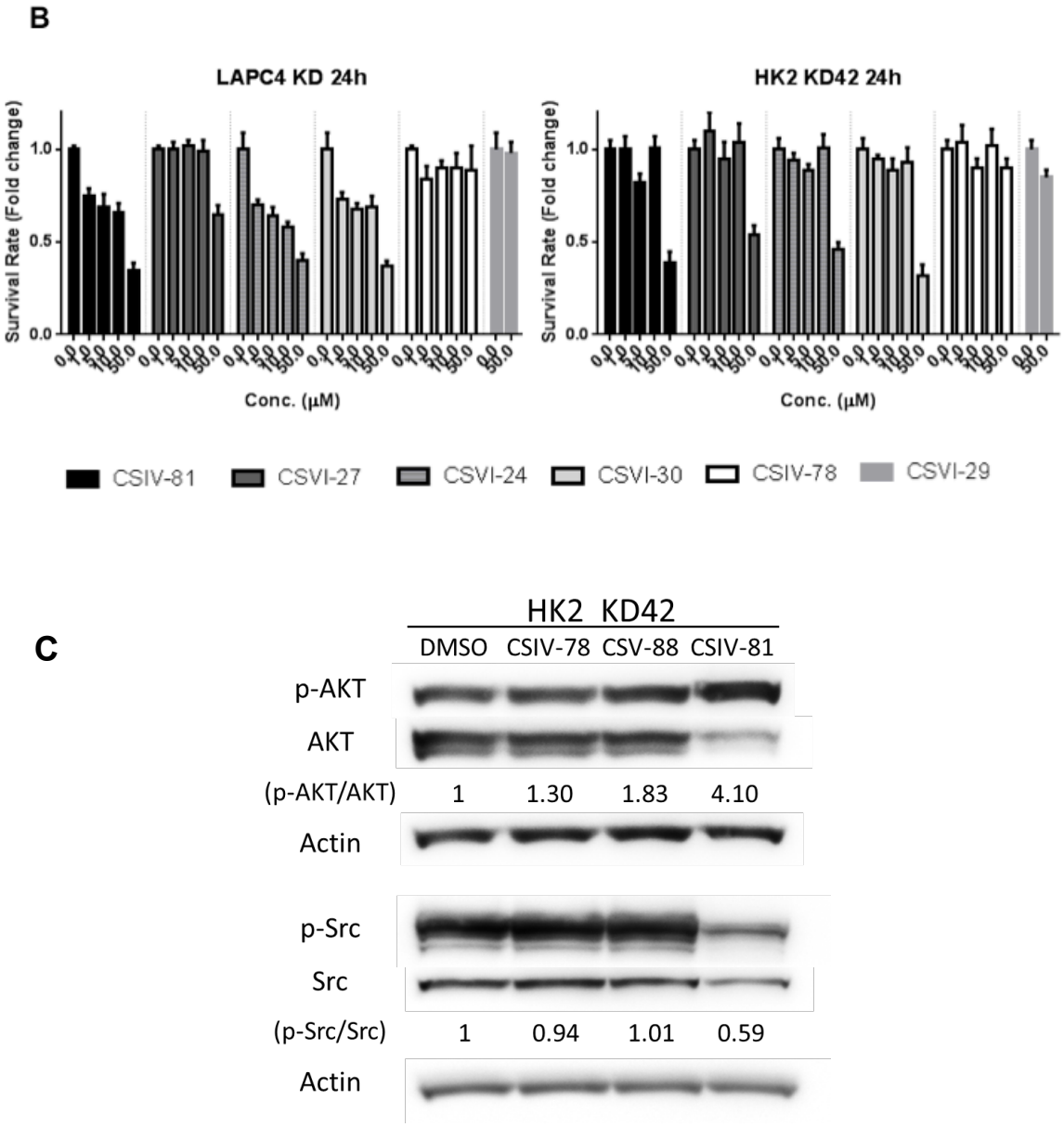
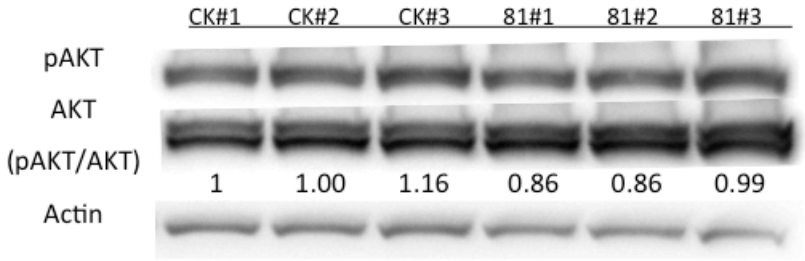


Figure 2 The *in vivo* activities of CSIV-81. Three animals per group were injected with LAPC4KD ($2X10^6$ cells/site) subcutaneously. After tumor became palpable, minipump containing 10mg/kg CSIV-81 was implanted nearby tumor site for 24 hrs then tumors were excised to prepare cells lysates subjected to western blot analyses.


CK#1
CK#2
CK#3
81#1
81#2
81#3
pAKT
AKT
(pAKT/AKT)
1
1.00
1.16
0.86
0.86
0.99
Actin